REMARKS/ARGUMENTS

Status of the Claims

Upon entry of the present amendment, claims 1-2, 4-11 and 17-19 are pending. Claim 3 is canceled without disclaimer or prejudice to renewal. No new matter is added by the present amendments, and the Examiner is respectfully requested to enter them.

Rejection under 35 U.S.C. § 112, second paragraph

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Applicants do not agree with the Examiner's position. However, in the interest of furthering prosecution, Applicants have cancelled claim 3. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Rejection under 35 U.S.C. § 103(a)

Claims 1-11 and 17-19 stand rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious over WO 02/06450 ("Darimont") in view of U.S. Patent No. 7,015,037 ("Furcht"). This rejection is respectfully traversed for the reasons discussed below.

The Examiner has the burden of presenting a *prima facie* case of obviousness. For an invention to be obvious under 35 U.S.C. § 103(a) requires consideration of the factors set forth in *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966), including an analysis of the scope and content of the prior art and the differences between the claimed subject matter and the prior art. Indeed, "rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *See*, *KSR Int'l Co. v. Teleflex Inc.*, 500 U.S. 398, 418 (2007), quoting *In re Kahn*, 441 F.3d 997, 988 (Fed. Cir. 2006)).

Here, Applicants respectfully maintain that the Examiner's articulated reasons for alleged obviousness do not have sufficient rational underpinnings to support a legal conclusion of obviousness. No *prima facie* case of obviousness has been established.

The present invention is directed to a population of primary cultured preadipocytes. That is, the present population of ***primary cultured preadipocytes*** are not immortalized cell lines or a cell population that has been manipulated to have enhanced replicative potential (and which may be immortalized or transformed).

In direct contrast, Darimont discloses the provision of human adipose ***cell lines***. For example, the title of Darimont recites "PRE-ADIPOSE CELL LINES" (emphasis added) Darimont defines their invention as "new human pre-adipose cell lines capable to differentiate to adipose cells." *See*, Darimont at the abstract and page 1, lines 1-2 (emphasis added). Darimont goes on to state that:

> **An object of the present invention resides therefore in providing means to further investigate the role of white adipose tissue in the body. Another object of the invention is to provide means that allows an investigation of the effect of novel drugs or food ingredients on white adipose tissue. The above object has been solved by providing novel pre-adipocyte cell lines derived from white adipose cells that have the capability to differentiate into mature white adipose cells, while exhibit essentially the same cellular properties as do normal white adipose cells.**

Darimont at page 3, lines 10-17 (emphasis added). *See also*, claim 1. The Examiner acknowledges that Darimont describes that establishment of human preadipose cell lines on page 4 of the present Office Action.

Moreover, the cell lines described in Darimont are immortalized. Darimont describes at page 5 line 31 bridging to page 6, line 29 methods for preparing the pre-adipose cell lines, reporting that "the preadipocytes thus obtained are immortalized. … a selection is made for cells positively immortalized… the cells shown to be positively immortalized by the introduction of the vector are tested for their ability to differentiate into mature white adipose cells. (emphasis added). Claim 4 of Darimont recites a "method for preparing a pre-adipose cell line…which comprises the steps (a) separating cells…; (b) de-differentiating and proliferating the cells…; (c) immortalizing a pre-adipose clone…; (d) selecting for immortalized cells, and (e) selecting for cells capable to differentiate into white adipose cells" (emphasis added).

In addition to performing a "de-differentiation" step, Darimont further selects for immortalized cells and prepares cell lines. Thus, Darimont provides a method for obtaining an

adipose "cell line", and not for obtaining a population of primary cultured preadipocytes, as claimed in the present application. Even if the method of Darimont could generate preadipocytes as an intermediate before achieving the immortalized adipose cell line, there would be no reason for the skilled person would abort cell processing and use the intermediate as a substitute for stromal cell preadipocytes in Furcht, as proposed by the Examiner.

An analogous argument applies to the combination of U.S. Patent Application Publication 2005/0008621 ("Kirkland," not relied upon in the present Office Action) with Furcht.

Kirkland describes the production of preadipocytes engineered to have enhanced replicative potential be expressing telomerase reverse transcriptase. Kirkland notes that the use of primary culture has serious disadvantages. In paragraph [0008], Kirkland states that it is difficult to isolate preadipocytes from other fibroblast-like cells and that large amounts of fat tissue are required because preadipocytes constitute only a small percentage of total fat tissue. In addition, Kirkland notes that primary cultures have a limited life span and lose adipogenic potential in culture.

In paragraph [0009], Kirkland goes on to state that:

> **Further problems are associated with studies of human primary cells. In particular, human preadipocytes are technically difficult to isolate and culture and only very few laboratories are capable of routinely culturing these cells. As with other human cell types, the capacity of human preadipocytes to replicate declines gradually as they are passaged, but their capacity to differentiate into fat cells declines rapidly. Since it is therefore necessary to use primary or early passage cultures to study adipogenesis, these cells are very expensive as initial collection and isolation account for most of the cost. Culture purity can also be an issue since cultures cannot be derived from single cells and still maintain capacity to differentiate. Thus, coupled with limited replicative potential, makes it impractical to manipulate and study adipogenic function and mechanisms using, for example, stable transfection approaches. Furthermore, fat specimens are difficult to obtain in quantity, particularly from lean subjects, very old subjects, and fat depots that are difficult to access.**

Accordingly, Darimont does not teach or suggest the present invention and Kirkland teaches away from the present invention, which utilizes primary cultured preadipocytes. The adipose "cell line" described in Darimont and/or Kirkland is an

"immortalized" cell line, and is distinct from the primary cultured preadipocytes cell populations of the present invention. The immortalized cell lines of Darimont and Kirkland carry significant problems that prevent them from being applied to transplantation. In particular, there is the concern of cancerous transformation and uncontrollable proliferation *in vivo*. Considering any practical application to therapy (*e.g.*, use as an implant composition for gene therapy as claimed herein), such cancerous transformation or uncontrollable proliferation *in vivo*, or even the immortalized nature of the cells, is quite dangerous because these characteristics can lead to the continued increase in the number of cells that carry the therapeutic gene, leading to an unstable and increasing supply of therapeutic protein in the body. If the therapeutic gene is an insulin gene, such increasing expression/supply of the insulin protein in the body could cause hypoglycemia which could, in the worst case, kill a patient.

In contrast, the primary cultured preadipocytes achieved in the present invention are not only long-lasting but also stably express a foreign gene after the preadipocytes are implanted in a living body. No substantial rise in expression level of the foreign gene was observed, and as disclosed in the specification, variance in expression level was as little as three-fold under optimal conditions. *See, e.g.*, page 36 of the specification, stating:

> **Fig. 13 shows the result of a long-term examination of blood AP activity in the implanted mice of Fig. 12(A), and in mice receiving an implant by a variety of other methods. In the group implanted with PLAP-transfected cells, a clear increase of blood AP activity was confirmed for all implantation sites and implantation methods. Blood AP activity was maintained for a long period, and in particular, stable AP expression was observed for one year during the Dif/Sc group testing period (the group described in Fig. 12(A)). Continuous AP production was also confirmed for the other implantation methods, all during the examination period (316 days for the ip group, 54 days for the fat group, 225 days for the Sc group, 317 days for the Mat/Sc group, and 314 days for the two pre-fix groups). The peak of activity observed within one week of implantation was highest in the ip group. The highest values were then in the order of Sc>fat>Dif/Sc>pf-dif>pf-gr>Mat/Sc. The range of variation after implantation was observed as a ratio between the activity after 13 weeks and the peak activity, which can be compared in all groups. Variance was smallest, approximately three-fold, in the two pre-fix groups, approximately five-fold in the ip, Dif/Sc, and Mat/Sc groups, and approximately ten-fold in the Sc and fat groups. The peak value**

immediately after implantation, and the range of variation after implantation differed for each implantation method. Any of these methods can thus be used according to the characteristics of the gene product used, the pathologic characteristics, and the simplicity of the technique. This showed that implantation of primary cultured adipocytes, to which genes were stably introduced ex vivo, can be performed by a variety of methods, and that long-term stable *in vivo* gene expression is possible after implantation." (emphasis supplied).

This long-lasting and quite stable expression of a foreign gene is a surprisingly beneficial characteristic of the claimed primary cultured preadipocytes, which characteristic could not be predicted from Darimont or Kirkland, either alone or in combination with Furcht.

At the time the June 18, 2002 priority date of the present application, there was a common understanding in the art that nearly one year long-term expression of a transgene by cells *in vivo* was extremely difficult (or not possible) even by using a retrovirus vector (RV) or an adeno-associated virus (AAV) vector. RV and AAV were generally known as vectors achieving good expression of a foreign gene. *See, e.g.*, Bestor, *J. Clin Invest.* (2000) 105(4):409-11 and Wei, *et al.*, *Gene Ther.* (1999) 6(5):840-4.[1]

Bestor states that "[l]ong-term expression - on the order of years, rather than weeks does not seem to have been demonstrated for any retroviral vector" (emphasis supplied). *See*, Bestor at page 410, left column, line 10. This statement clearly supports that, even if retroviral vectors and adeno-associated viral vectors were known as vectors to achieve good expression of a foreign gene, long-term expression (such as for one year or more) of a foreign gene *in vivo* was against the technical common knowledge at the time of filing the present application.

In connection with the above-cited quotation, Bestor cites to Wei. Wei used retroviral vectors and primary fibroblasts, and found that the retroviral LTR is one possible target of silencing of a transgene and that use of an internal Fibronectin promoter between the retroviral LTRs permitted prolonged expression of several months after engraftment of infected cells. According to Wei, viral vectors using the virus's LTR (the present invention) would not have been predictable to achieve foreign gene expression "on the order of years."

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[1] Bestor, *J. Clin Invest.* (2000) 105(4):409-11 and Wei, *et al.*, *Gene Ther.* (1999) 6(5):840-4 are attached as Exhibits A and B, respectively.

According to Bestor, achievement of foreign gene expression "on the order of years" was against the common technical knowledge at the time of filing the present application, even if an attempt to prolong expression, such as by using promoter other than the virus's LTR to avoid silencing, was made. Therefore, the fact that the primary cultured preadipocytes of the present invention achieved stable expression of a foreign gene for as long as a whole year was not predictable from the art. Accordingly, the claimed invention is not obvious over Darimont or Kirkland, either alone or in combination with Furcht.

Applicants' data finds further support in post-filing date publications. For example, Spalding, *et al*, *Nature* (2008) 453:783-787[2] reports that adipocytes are quite stable cells in a living body, having a half-life of about 8.3 years. *See*, Spalding at page 785, right column. At the time the present application was filed, it was not known how long adipose cells including preadipocytes could persist in a living body. At the time of filing date of the present application, knowledge about the life span and renewal of adipose cells in a body was scarce (it was suggested that adipose cells reached a plateau in their number early in life and gradually gained and/or lost their cell volume thereafter). Despite such a poor understanding of adipose cells in the body by those of skill in the art, the inventors conducted dedicated studies and achieved long-term, stable expression of a foreign gene in a cell population of primary cultured preadipocytes.

Accordingly, in view of the foregoing, no *prima facie* case of obviousness has been established. Regardless, Applicants have rebutted any allegations of obviousness with a showing of surprising or unexpected properties. M.P.E.P. § 2145. As discussed above, the primary cultured preadipocytes of the present invention possess numerous advantages for the delivery of a secreted therapeutic protein over prior known cell delivery systems. They are not immortalized and have not undergone cancerous transformation. Thus, unlike other cell delivery systems, the primary cultured preadipocytes are safe. In addition, the primary cultured preadipocytes surprisingly achieve effectively stable expression of the therapeutic protein expressed from the foreign gene, and for extended periods of time. In view of the long half-life

[2] Spalding, *et al*, *Nature* (2008) 453:783-787 is attached as Exhibit C.

of adipocytes in a living body, adipocytes are clearly potent as an *in vivo* "factory" of a therapeutic protein encoded by a foreign gene.

Applicants respectfully remind the Examiner that the present primary cultured preadipocytes additionally possess the following advantageous characteristics, discussed in the Specification at page 1, line 35 through page 2, line 23. Such characteristics are not disclosed or suggested in the context of the cells of Darimont, Kirkland, or Furcht:

(1) preadipocytes are cells having hormone producing function and thus are suitable as secretary organs;

(2) adipocytes can be easily collected and are suitable for subcutaneous implantation, and techniques relating to their extirpation are developed;

(3) isolated primary cultured preadipocytes actively proliferate, even *in vitro*;

(4) preadipocytes are likely to stay in a limited area after implantation, and thus, are beneficial when considering extirpation after implantation;

(5) preadipocytes produce angiogenetic factors, and thus, a high level of engraftment can be expected after implantation;

(6) preadipocyte extirpation or implantation has a small impact on the human body; and

(7) preadipocytes are widely recognized as superfluous and obstructive, and consent for their collection may be obtained easily.

The primary cultured preadipocytes claimed herein are also advantageous in that gene transfer by using "retrovirus vector" is possible. As one embodiment of the present invention, a foreign gene is transferred to the claimed population of preadipocytes by using a retrovirus vector. While the resulting population of preadipocytes possesses the identified beneficial effects of stable and long-lasting foreign gene expression, such cells are only obtainable by introducing a foreign gene into primary cultured preadipocytes because retrovirus vectors cannot introduce genes into non-proliferating cells, such as fully-differentiated mature adipocytes.

In summary, the present primary cultured preadipocytes are beneficial cells for the purpose of preparing implant composition for gene therapy in that they are easy to collect

without placing large burden on a patient/subject and thus are suitable to provide autologous cell population; proliferate well *in vitro*; are easy to control differentiation so as to prepare an implant composition with uniformly differentiated cells; and are likely to stay in a limited area after implantation and thus are easy to extirpate after implantation. Cells satisfying these conditions and that at the same time provide long-lasting and stable foreign gene expression did not exist until Applicants invented the presently claimed primary cultured preadipocytes. Therefore, the primary cultured adipocytes of the invention fulfill an unmet need for better methods of delivering therapeutic proteins to a patient.

Accordingly, because the present populations of primary cultured preadipocytes are not obvious over Darimont or Kirkland, either alone or in combination with Furcht, the Examiner is respectfully requested to withdraw this rejection.

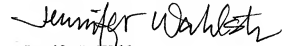
CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Further, the Commissioner is hereby authorized to charge any additional fees or credit any overpayment in connection with this paper to Deposit Account No. 20-1430.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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